CELLULAR MORPHOLOGY AND ARCHITECTURE DURING EARLY MORPHOGENESIS OF THE ASCIDIAN EGG: AN SEM STUDY

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Classical studies of ascidian embryogenesis, especially Conklin's (1905) excellent description, have established that ascidian gastrulation begins during seventh cleavage (see Berrill, 1955, Table II). That is, the most dramatic changes in cellular morphology and architecture occur in the embryo composed of about 100 to 200 cells. This number is quite small, compared to that of an early gastrula of other animals; the early gastrula of sea urchins consists of about 800 cells (Hinegardner, 1967), and the number of constituent cells of an amphibian gastrula is about 13,000 (Hara, 1977).

Scanning electron microscopy (SEM) is a powerful method of accurately and efficiently representing the complex three-dimensional morphology of rapidly growing embryonic systems (Waterman, 1972). Not only does SEM provide abundant opportunities for visualizing the surface features of developing embryos, but also observations with SEM of cut or fractured surfaces can provide information regarding the cellular architecture of embryos and the internal surface topography of hollow structure. During the past few years, several workers, utilizing SEM, have analysed morphological changes of the cell surface during gastrulation in a variety of animals (Monroy, Baccetti and Denis-Donini, 1976; Keller and Schoenwolf, 1977; Turner and Mahowald, 1977). The present paper deals with cellular morphology, contact and arrangement in the blastula, gastrula and neurula stages of the ascidian, *Halocynthia roretzi,* as examined by SEM. The observations have emphasized several characteristic features of the early morphogenesis of the ascidian egg.

MATERIALS AND METHODS

Naturally spawned eggs of the ascidian, *Halocynthia roretzi,* were reared in filtered sea water at room temperature (15–16° C). The embryos were fixed either in 2.0% glutaraldehyde in 0.1 M cacodylate in sea water (pH, 8.1) or in 1.0% osmium tetroxide in 0.1 M cacodylate in sea water, and rinsed in the buffered sea water. After removal of the chorion, the embryos were dehydrated through a graded series of ethanols. To reveal internal details of the embryos, selected stages were dissected in the appropriate plane with fine forceps while they were in 70% ethanol. All specimens were dried by the critical point technique, using CO_2 with amyl acetate as the transition fluid, with a Hitachi HCP-1 apparatus. The dried embryos were mounted on aluminum stubs, coated with a thin layer of gold utilizing a Giko IB-3 ion coater, and examined with a Hitachi S-310 SEM.

In order to supplement and substantiate the results of the examination with SEM, attentive observations on living materials were also carried out.

The 64-cell stage

The outer surface of the embryo examined with the SEM was essentially smooth (Figs. 2 and 3). For a while after cell division, thin cytoplamic processes like microvilli could be seen on the cell surface bordering the interstices between the daughter cells. The two or three polar bodies were present near the animal pole (Fig. 2).

Examinations of the dissected embryos revealed the formation of the blastocoel, which was enclosed by a layer of cells (Fig. 1). The cells of both hemispheres were of nearly equal height at this stage, those of the vegetal (dorsal) hemi-sphere being slightly taller than those of the animal (ventral) hemisphere (Fig. 1a). On the cell surface bordering the blastocoel numerous cell processes were extended from cells onto adjacent cells (Fig. 1b). The cell processes or filopodia were about 0.2 to 0.5 μm in diameter and varied from 2 μm to over 10 μm in length (Fig. 1b). The blastocoel was observed for a while after cell division. By the time the divided cells adhered closely together, the blastocoel had disappeared.

Gastrulation

In the egg of *H. roretzi*, similar to those of other ascidians, gastrulation began during the seventh cleavage. The seventh cleavage began on the anterior part of the dorsal (vegetal) hemisphere. After a while, divisions of all the cells of the ventral (animal) hemisphere took place synchronously. After these divisions, changes in cell shape and cellular architecture of the embryo progressed rapidly. The outer surface of the dorsal cells became smaller than that of the vegetal ones; the meso- and endoderm cells at the dorsal hemisphere grew slightly long and narrow, while the ectoderm cells at the ventral hemisphere became broad and flat (Figs. 2, 3, 6; also, compare Fig. 6 to Fig. 1). At the same time a layer of the ectoderm cells and that of the meso- and endoderm cells became adhered closely together (Fig. 6). Thereafter, the blastocoel could no longer be observed (Figs. 6–8).

The initiation of gastrulation was first detected by the reduction of the surface area of the two cells of the dorsal hemisphere (Fig. 3). The two cells were $A^{7.1}$ and $A^{7.1}$ of Conklin's (1905) nomenclature. Examinations of the dissected early gastrula along the midsagittal plane revealed that the two cells changed in shape from tall columnar to wedge-shaped cells (Fig. 6). Their apices became constricted and their bases enlarged (Fig. 6). Viewing from the dorsal pole, the endoderm cells seemed to invaginate, led by the two cells (Fig. 4). In the dis-sected early gastrula the layers of the ectoderm and endoderm cells adhered closely together, and the ectoderm layer began to fold the endoderm layer (Figs. 6–8). By further epiboly or enfolding movement of the ectoderm cells coinciding with a folding down of the endoderm cells, the gastrocoel was formed (Figs. 8, 11)

The blastopore of the middle gastrula was of a peculiar form, wide in the anterior and narrow in the posterior (Fig. 5). This might be due to different outgrowths or epibolic movements of the ectoderm cells. The outgrowth began in
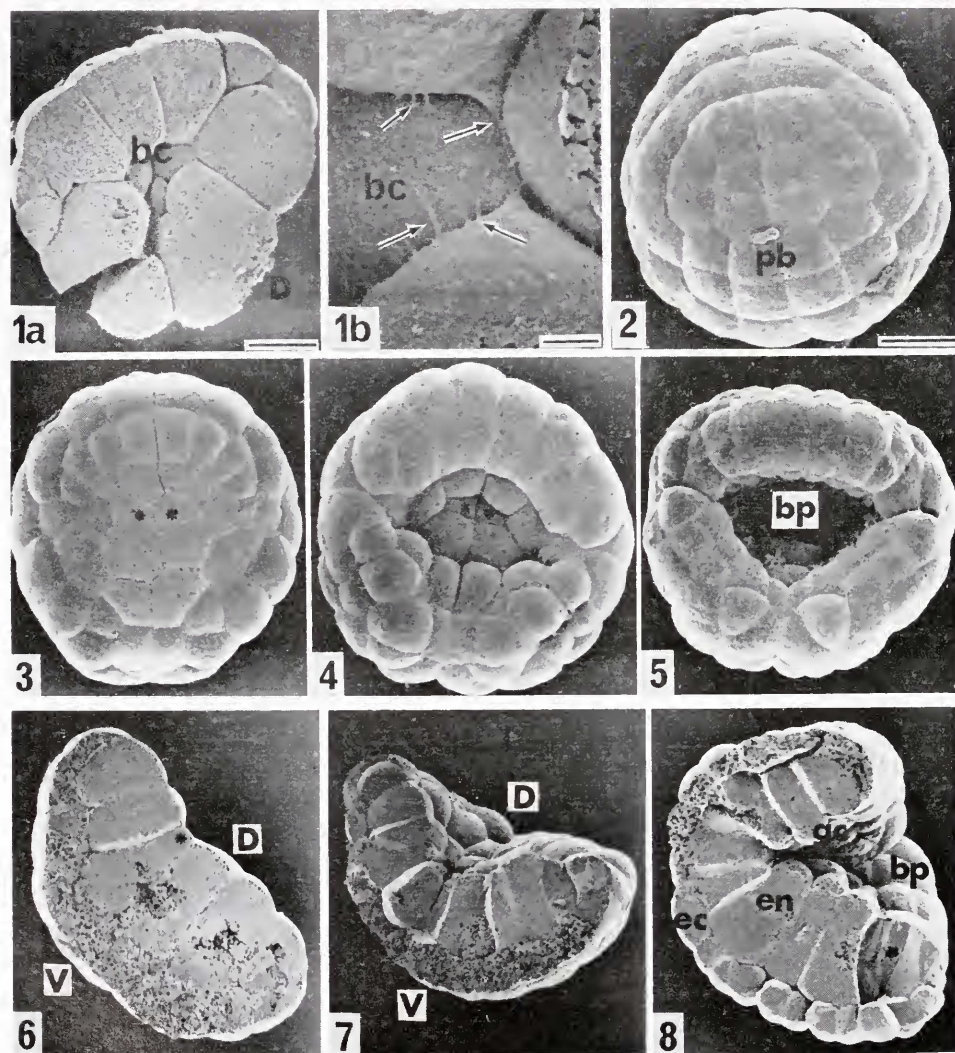
FIGURE 1. Scanning electron micrograph (SEM) of the dissected 64-cell stage showing the formation of the blastocoel (bc). (1a) The cells of both hemispheres are of nearly equal height (D, dorsal side; V, ventral side). Bar, 50 μm. (1b) Cell surface bordering the blastocoel. The cells extend cytoplasmic processes or filopodia onto adjacent cells (arrows). Bar, 5 μm. The embryo is shown with the anterior end up and the posterior end down in all figures.

FIGURE 2. Early gastrula stage viewed from the ventral (animal) pole. The ectoderm cells have divided seven times (pb, polar bodies). Bar, 50 μm.

FIGURE 3. Initial gastrula stage viewed from the dorsal (vegetal) pole. Note the reduction of the surface area of the endoderm cells, especially the two cells marked by asterisks.

FIGURE 4. Early gastrula stage viewed from the dorsal pole. Emboly of the endoderm cells begins. Asterisks show the two leading cells. Several meso- and endoderm cells are dividing.

FIGURE 5. Middle gastrula stage viewed from the dorsal pole. The blastopore (bp) is

the front of the anterior quadrants and progressed rapidly. At the right and left sides of the blastopore the outgrowth was slow, while at the posterior pole the outgrowth was further delayed (Figs. 4, 5). The gastrocoel cavity was wide in the anterior, narrowing towards the posterior (Fig. 8). The cell surface bordering the gastrocoel was generally round and smooth without cell processes (Figs. 8, 11). At the late gastrula stage, the blastopore shifted posteriorly by rapid epibolic movement of the anterior lip (compare Fig. 5 to Fig. 9). During the succeeding phase of gastrulation, the blastopore shifted back from posterior to dorsal by the outgrowth of the posterior lip, as well as that of right and left side lips (compare Fig. 9 to Fig. 10). Coincident with the shift, the blastopore became narrow and oval (Fig. 10). Examinations of the dissected embryos showed that gastrulation was almost accomplished at this stage (Fig. 11).

At the middle gastrula stage the eighth division of the ventral ectoderm cells occurred almost synchronously. The ninth division of the ectoderm cells took place at the late gastrula stage. The middle gastrula was nearly circular in outline viewed from the dorsal or ventral pole (Fig. 5). At the late gastrula stage the embryo became elongated and egg-shaped, the posterior end being somewhat narrower than the anterior (Fig. 10).

The process of closure of the blastopore temporally overlapped in part with that of neurulation.

Neurulation

The neural tube formation of the ascidian embryo progressed in a similar fashion to that of vertebrates (Schroeder, 1970; Karfunkel, 1974). The neural plate cells were distinguishable on the mid-anterior part of the dorsal side of the late gastrula (Figs. 9, 10). They were flat in shape and consisted of several transverse rows of cells. Neural tube formation proceeded from the posterior end to the anterior end (Figs. 12, 14). Cells were heaped in a mass at the anterior end of the neural fold (Fig. 12). The neural folds of the lateral edges of the neural plate rolled toward the midline. The neural plate cells underwent a gradual elongation in the early stages of neurulation, and then changed in shape from columnar to wedge-shaped cells, the apices of which became narrow (Fig. 13). When the folds met at the midline, the plate was folded into a tube (Fig. 14). The ectoderm lateral to the neural plate was also carried to the midline, and formed the epidermis covering the neural tube.

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of a peculiar form, wide in the anterior and narrow in the posterior. This may be due to different outgrowths of ectoderm cells.

FIGURE 6. Dissected initial gastrula stage along the midsagittal plane showing that the cells of both hemispheres adhere very closely together. The ectoderm cells of the ventral hemisphere (V) become shorter than the meso- and endoderm cells of the dorsal hemisphere (D). The invaginating cell becomes wedge-shaped (asterisk).

FIGURE 7. Dissected early gastrula stage along the midsagittal plane. The cells of the ventral hemisphere (V) begin to enfold those of the dorsal hemisphere (D).

FIGURE 8. Dissected middle gastrula stage along the midsagittal plane. By further enfolding movement of the ectoderm cells (ec), the gastrocoel (gc) is formed (bp, blastopore; en, endoderm cells).
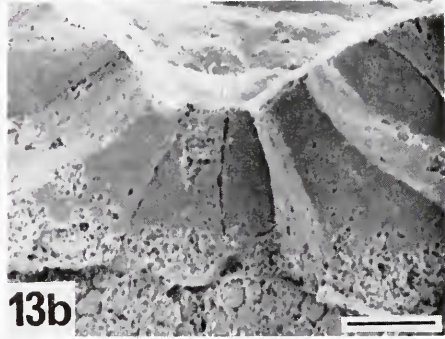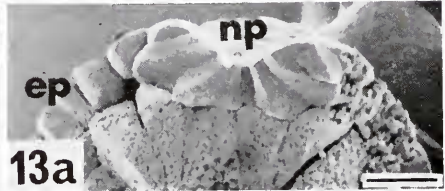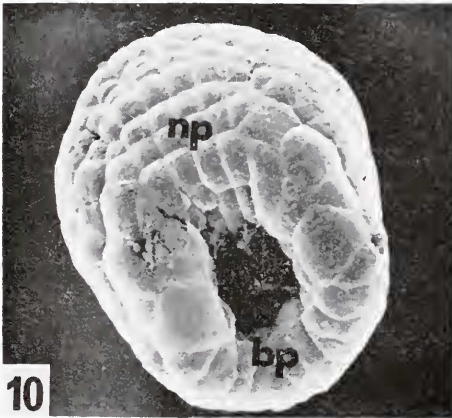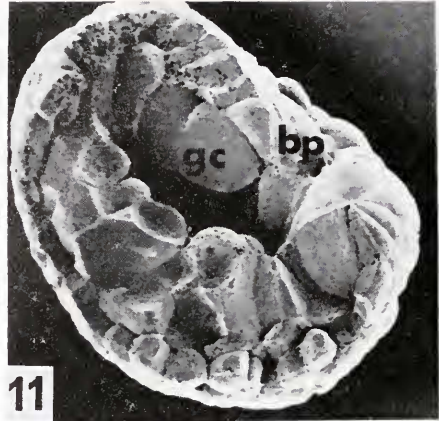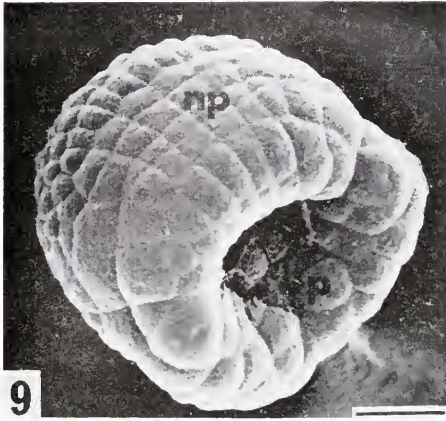
FIGURE 9. Late gastrula stage viewed from the dorsal side. The blastopore (bp) has shifted from the mid-dorsal portion of the embryo to the posterior portion. The neural plate cells (np) are distinguishable. Bar, 50 μm.

DISCUSSION

Early morphogenesis, including gastrulation and neurulation, of animal eggs is a phenomenon of many integrated cell movements, each of which depends directly or indirectly on every other (Trinkaus, 1969). Morphogenetic movements always involve deformation of constituent cells of the system. Based on accurate observations on living eggs and embryos, Conklin (1905) suggested two major factors for ascidian gastrulation; the first is the change in shape of the cells of the animal and vegetal hemispheres, and the second is the overgrowth of the marginal cells of the animal hemisphere. The present observations with SEM have fairly confirmed these two factors. The change in shape of the cells during gastrulation and the enfolding movement of the ectoderm cells can easily be imagined by comparing SEM photographs of dissected gastrulae. Another feature of the cell architecture during gastrulation of ascidian eggs may be the close adherence of the layer of the ectoderm cells and that of the meso- and endoderm cells. The blastocoel was observed in the dissected blastula for a while after cell division. However, by the time the divided cells adhered closely together, the blastocoel had disappeared. Pseudopodia observed on the cell surface bordering the blastocoel seem to contribute to cell-to-cell adhesion.

Mancuso (1973), utilizing transmission electron microscopy, has observed ultrastructural characteristics of the constituent cells in ascidian gastrula and neurula, and revealed bundles of microtubles at the peripheral, external cytoplasm of the neural blastomeres. At the early gastrula stage the endoderm cells changed in shape from columnar to wedge-shaped cells. Similarly, the neural plate cells changed in shape from flat, through columnar, to wedge-shaped cells. These changes in shape might be caused by a cytoplasmic system acting at the surface of wedge-shaped cells. It is expected that cytoplasmic contracting systems such as microfilaments act at the surface of the wedge-shaped cells.

In conclusion, the present observations with SEM of the ascidian morphogenesis has fairly confirmed the results of descriptive studies of ascidian embryogenesis.

FIGURE 10. Neural plate stage viewed from the dorsal pole. The embryo becomes elongated and egg-shaped. The blastopore (bp) becomes narrow and oval. Neural plate cells (np) are flat in shape and consist of several transverse rows of cells.

FIGURE 11. Dissected neural plate stage along the sagittal plane. Enfolding movement of the ectoderm cells has nearly completed (bp, blastopore; gc, gastrocoel).

FIGURE 12. Middle neurula stage viewed from the dorsal pole. The neural tube formation takes place from the posterior end of the embryo to the anterior end. At the anterior end of the neural fold (nf) cells are heaped in a mass.

FIGURE 13. (13a) Dissected early-to-mid neurula stage along the transverse plane showing rolling-up of the neural plate cells (np) (ep, epidermis). Bar, 30 μm. (13b) Higher magnification of the neural plate cells showing close adherence of the cells to one another. They are wedge-shaped. Bar, 10 μm.

FIGURE 14. Late neurula stage viewed from the dorsal side. The folds of lateral edges of the neural plate meet at the midline and then the plate is folded into tube (nt, neural tube).

SUMMARY

1. Cellular morphology and architecture during early morphogenesis of the ascidian embryo were examined by SEM.

2. The outer surface of the embryo was essentially smooth. The blastocoel could be seen in the dissected blastula. On the cell surface bordering the blastocoel, numerous pseudopodia extended from cells onto adjacent cells. These pseudopodia were suggested to contribute to cell-to-cell adhesion.

3. Before the initiation of gastrulation, a layer of the cells of the animal (ventral) hemisphere and that of the cells of the vegetal (dorsal) hemisphere adhered closely together. The blastocoel could no longer be observed.

4. The gastrulation began during the seventh cleavage. The gastrocoel was formed by a folding of the two layers of the cells. Examinations of the dissected gastrulae suggested two cooperative forces for the gastrulation: first, the epibolic or enfolding movement of the ventral ectoderm cells and secondly, the change in shape of the constituent cells.

5. The neural tube formation progressed in a similar fashion to that of vertebrates.

LITERATURE CITED

BERRILL, N. J., 1955. *The Origin of Vertebrates.* Oxford Univ. Press, London.

CONKLIN, E. G., 1905. The organization and cell-lineage of the ascidian egg. *J. Acad. Natl. Sci.,* **13** : 1–119.

HARA, K., 1977. The cleavage pattern of the axolotl egg studied by cinematography and cell counting. *Wilhelm Roux's Arch. Dev. Biol.,* **181** : 73–87.

HINEGARDNER, R. T., 1967. Echinoderms. Pages 139–155 in *"Methods in Developmental Biology",* F. H. Wilt and N. K. Wessells, Eds., Thomas Y. Crowell Comp., New York.

KARFUNKEL, P., 1974. The mechanisms of neural tube formation. *Int. Rev. Cytol.,* **38** : 245–271.

KELLER, R. E., AND G. C. SCHOENWOLF, 1977. An SEM study of cellular morphology, contact, and arrangement as related to gastrulation in *Xenopus laevis. Wilhelm Roux's Arch. Dev. Biol.,* **182** : 165–186.

MANCUSO, V., 1973. Ultrastructural changes in the *Ciona intestinalis* egg during the stages of gastrula and neurula. *Arch. Biol.,* **84** : 181–201.

MONROY, A., B. BACCETTI, AND S. DENIS-DONINI, 1976. Morphological changes of the surface of the egg of *Xenopus laevis* in the course of development. III. Scanning electron microscopy of gastrulation. *Dev. Biol.,* **49** : 250–259.

SCHROEDER, T. E., 1970. Neurulation in *Xenopus laevis.* An analysis and model based upon light and electron microscopy. *J. Embryol. Exp. Morph.,* **23** : 427–462.

TRINKAUS, J. P., 1969. *Cells into Organs.* Prentice-Hall Inc., New Jersey.

TURNER, F. R., AND A. P. MAHOWALD, 1977. Scanning electron microscopy of *Drosophila melanogaster* embryogenesis. II. Gastrulation and segmentation. *Dev. Biol.,* **57** : 403–416.

WATERMAN, R. E., 1972. Use of the scanning electron microscope for observation of vertebrate embryos. *Dev. Biol.,* **27** : 276–281.